trum was identical with that of the above polymer. Uv (THF): λ_{max} ($\epsilon \times 10^3$) 317 (9.01), 300 (9.08), 287 sh (8.50), 267 sh (6.7). GPC analysis gave $\bar{M}_n = 2.24 \times 10^4$, $\bar{M}_w = 6.7 \times 10^4$, MWD = 3.16.

Poly[1-(4′-vinylbenzyl)isoquinoline] (18). To a refluxing solution of 1.00 g (2.66 mequiv) of **17** (from run 2 above), 0.60 g of water, 3.5 g of ethoxyethanol, and 21.5 g of THF was added slowly 0.32 g (4.9 mmol) of 87% KOH. After 3.2 hr of refluxing the mixture was poured into water and filtered. The crude solid was precipitated from THF into water, washed with methanol, and dried to a weight of 0.56 g (86%). Two more precipitations left 0.33 g. Anal. Calcd for $C_{18}H_{15}N$: C, 88.13; H, 6.16; N, 5.17. Found: C, 88.6; H, 5.94; N, 5.63. The infrared spectrum (Figure 3b) is virtually identical with that of **7**(Figure 3a) prepared by hydrolysis of **6**. Uv (THF): λ_{max} ($\epsilon \times 10^3$) 321 (3.25), sh 316 (2.24), 308 (2.50), sh 280 (4.09), 272 (5.07), 267 (4.67). GPC analysis yields $\bar{M}_n = 1.20 \times 10^4$, $\bar{M}_w = 5.60 \times 10^4$, MWD = 4.66.

Poly{styrene-*co*-[1-(4′-vinylbenzyl)-2-benzoyl-1,2-dihydroisoquinaldonitrile]} (19). A solution of 3.76 g (10.0 mmol) of **14** (2.66 g of solid, mp 146–150°; 1.10 g of solid, mp 140–145°), 1.04 g (10.0 mmole) of deinhibited styrene, 22.4 mg of AIBN, and 45 ml of dry benzene was maintained at 61° in a sealed ampule for 97 hr. The solid initially obtained by precipitation into methanol was precipitated from THF into methanol twice and dried to a weight of 2.97 g (63%). Anal. Calcd for $C_8H_8 \cdot 1.1C_{26}H_{20}N_2O$: C, 84.82; H, 5.84, N, 5.95. Found: C, 84.72; H, 6.03; N, 6.02. The infrared spectrum is very similar to that of **17**. The ultraviolet spectrum has the following: λ_{max} ($\epsilon \times 10^{-3}$ calculated on isoquinoline equivalent basis) 317 (7.94) and 300 (8.23). GPC gave $\bar{M}_n = 2.35 \times 10^4$, $\bar{M}_w = 9.40 \times 10^4$, MWD = 3.99.

1-Allyl-2-benzoyl-1,2-dihydroisoquinaldonitrile (20). To a solution of 2.60 g (10.0 mmol) of **1**, 4.80 g (40.0 mmol) of allyl bromide, and 25 ml of dry DMF at 0° was added 0.40 g (10.0 mmol) of 57% sodium hydride. The solution was stirred for 0.5 hr and poured into 400 g of ice–water. A stream of air was used to evaporate the excess allyl bromide and then the solution was filtered, yielding 3.00 g (100%) of tan solid. Recrystallization from hexane-ethyl acetate gave large colorless crystals, mp 101.0–103.5°. Anal. Calcd for $C_{20}H_{16}N_2O$: C, 79.98; H, 5.37; N, 9.33. Found: C, 80.06; H, 5.40; N, 9.43. The ultraviolet spectrum (ethanol) has the following: λ_{max} (ϵ) 321 sh (1×10^4), 297 (1×10^4), 287 sh (1×10^4), 228 nm (3×10^4). NMR ($CDCl_3$): δ 2.98 (septet, AB part of ABX system, J_{AB} = 13 Hz, J_{AX} = 4.2 Hz, J_{BX} = −4.2 Hz), 2 H ($-CH_2-$)), 4.5–5.7 (m, 3 H ($-CHCH_2-$)), 5.35 (d, J = 8 Hz, 1 H (H_4)), 6.06 (d, J = 8 Hz, 1 H (H_3)), 6.5–7.4 (m, 9 H (arom)).

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Multifunctional Hydrolytic Catalyses. IV. The Catalytic Hydrolysis of *p*-Nitrophenyl Acetate by Copolymers Containing Complementary Functional Groups (Hydroxamate and Imidazole)

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ABSTRACT: Water-soluble bifunctional polymers which contained *N*-phenylacrylohydroxamate unit and 4(5)-vinylimidazole unit were prepared, and the catalytic hydrolysis of *p*-nitrophenyl acetate was studied at 30°C in 28.9% $EtOH-H_2O$. Under ordinary substrate concentrations (10^{-4}–10^{-5} *M*), the reaction followed the simple first-order kinetics, indicating the complete turnover of the catalytic group. At higher substrate concentrations, the time course of the reaction was biphasic and the hydrolysis was shown to proceed mainly via the formation and the subsequent decomposition of the acetyl hydroxamate intermediate. The acylation rate at the hydroxamate anion unit was affected only insignificantly by the presence of the imidazole unit, and was much more efficient than those at the imidazole site. In contrast, the decomposition of the acetyl intermediate was remarkably accelerated (up to 10^3-fold) by the introduction of the imidazole unit. Thus, the appropriate combination of the nucleophilic functions of complementary nature led to much enhanced turnover rates of the catalytic site.

It is generally believed that the cooperative action of several functional groups at the active site is crucial for the remarkable efficiency of enzyme catalyses, and, in fact, it has been shown that a combination of functional groups named the charge relay system catalyzes hydrolyses of esters and amides very efficiently in some serine proteases.[2a]

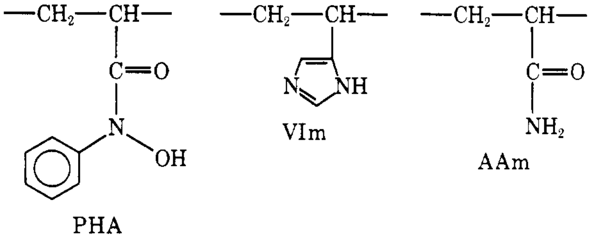
In much simpler systems, attempted observation of the multifunctional catalysis (polymeric[2b–4] and nonpolymeric

Table I
Copolymerization[a]

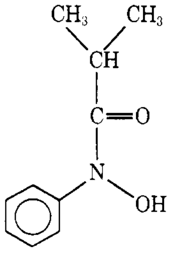
	Monomer, M			Time, min	Conversion, wt %	Copolymer composition, mol %			
Run	PHA	VIm	AAm			Method[b]	PHA	VIm	AAm
1	0.07	0.40	0.53	90	35	PT	5	35	60
						NMR	5	31	64
2	0.07	0.07	0.84	50	40	PT	3	7	90
						NMR	3	9	88
3	0.07	0.70	0.23	50	17	NMR	7	58	35
4[c]	0.10	0.90		100	20	NMR	13	87	
5	0.20	0.80		180	30	NMR	22	78	
6		0.10	0.90	20	40	PT		10	90

[a] 80°; benzene solvent; AIBN, 0.25 mol % of total monomer. [b] The copolymer composition was determined either by the potentiometric titration (PT) or by NMR spectroscopy (NMR). [c] 70°; benzene solvent; AIBN, 0.25 mol % of total monomer.

types[5]) of hydrolysis has met with limited success. We reported previously[6,7] that a copolymer containing a hydroxamate unit (PHA) and a N-alkylimidazole unit showed an enhanced catalytic activity in the hydrolysis of p-nitrophenyl acetate due to increased rates of decomposition of the acetyl hydroxamate intermediate. However, the deacylation rate was not large enough to give efficient turnover of the catalytic group. In the present study, we incorporated into polymer the 4(5)-vinylimidazole unit in order to attain further increase in the deacylation rate, because poly(4(5)-vinylimidazole) was reported to show accelerated deacylation behavior.[8] In fact it was found that the new bifunctional polymer gave higher catalytic efficiencies. A preliminary account of this paper[9] and related investigations in small-molecule systems[10,11] have been presented elsewhere.



PHA VIm AAm



model PHA

Experimental Section

Materials. p-Nitrophenyl acetate and acetyl N-phenylacrylohydroxamate (bp 100–110° (0.08 mm)) were prepared as described before.[12] Acrylamide was recrystallized from benzene: mp 83.5–84.5°. 4(5)-Vinylimidazole was obtained by thermal decarboxylation of urocanic acid according to the procedure of Overberger et al.[13] The fraction collected at 110–130° (0.1 mm) was shown to be of satisfactory purity and was used without further purification: yield 50–60%; mp 83° (lit.[13] bp 118° (0.3 mm), mp 83–84°).

Copolymerizations. Copolymerizations of acetyl N-phenylacrylohydroxamate (acetyl PHA), 4(5)-vinylimidazole (VIm), and acrylamide (AAm) were carried out in degassed ampoules using azobis(isobutyronitrile) as initiator, under the conditions specified in Table I. The precipitates were filtered, washed with acetone and reprecipitated from water and acetone. The polymer was dissolved in water, treated with excess hydrazine for 24 hr, and poured into acetone (90–97% recovery). Completion of deacetylation was confirmed by disappearance of an ir absorption at 1800 cm^{-1}.[12] The VIm-AAm copolymer was prepared in the same way, except for the hydrazine treatment.

The composition of the PHA-VIm-AAm terpolymer was determined by the potentiometric titration and NMR spectroscopy. The titration curves of the PHA and VIm units are not well resolved. Therefore, the potentiometric titration with 0.1 N NaOH was performed for the terpolymer solutions in 30 v/v % EtOH–H_2O with and without addition of hydrochloric acid. The combined content of the PHA and VIm units was obtained from the titration of the acidic solution, and only the PHA unit was titrated without addition of hydrochloric acid. The copolymer composition can be determined also from the relative peak areas of NMR spectra. NMR spectra of the terpolymer (in acidic D_2O) are composed of three peak groups: A, 8.6 ppm, imidazolium C-1 proton; B, 7.3–7.7 ppm, PHA phenyl proton and imidazolium C-4 proton: C, 2.3–3.2 ppm, methine and methylene protons.

The compositions obtained by these two methods show satisfactory agreements as shown in Table I. In the following kinetic calculations were employed preferentially the composition determined by the potentiometric titration.

The copolymers PHA-VIm-AAm-3, PHA-VIm-4, and PHA-VIm-5 (Table I, Run No. 3, 4, and 5, respectively) are soluble in 28.9 v/v % EtOH–H_2O in the acidic and alkaline region, but not soluble in the neutral region. Therefore, they were not employed as catalyst, though their compositions could be determined by NMR spectroscopy.

Determination of pK_a. The pK_a value of the PHA unit in the copolymer was determined from the uv absorption of the hydroxamate anion at 300 nm. Unfortunately, the VIm unit possesses weak absorption in this region (ϵ = 52 in 0.01 N NaOH and ϵ = 19 in 0.01 N HCl at 300 nm). This absorption was corrected by placing in the reference cell solutions of a VIm-AAm copolymer containing equimolar amounts of the VIm unit. The resulting absorption at 300 nm is solely attributed to the PHA unit: ϵ = 1980 in 0.01 N NaOH and ϵ = 770 in 0.01 N HCl. The pK_a value of the VIm unit in the copolymers was determined by the potentiometric titration.

The titration data were plotted according to the modified Henderson–Hasselbach equation[14]

$$\mathrm{p}K_a = \mathrm{pH} + n' \log \frac{1 - \alpha}{\alpha} \tag{1}$$

where α is the fraction of the dissociated PHA unit or of the neutral VIm unit.

The titration data are summarized in Table II.

Rate Measurements. All the reactions were performed at 30° in 28.9 v/v % EtOH–H_2O. The reaction of PNPA was followed by measuring the release of the p-nitrophenolate anion at 401 nm. The hydrolysis of the acetyl PHA group was followed by using the absorption at 300 nm of the hydroxamate anion formed.[12] In the case of the burst reactions, the 0.094-cm path length was adopted by the use of a spacer. Otherwise, a 1-cm quartz cell was used as such. Detailed procedures for the rate measurement were described before.[7] The reaction rates were calculated by using a programmable desk calculator (Compet 364P, Sharp Co. Ltd.) The least-squares treatment was carried out whenever possible. The correlation coefficient was better than 0.99 unless stated to the contrary.

Miscellaneous. All the UV measurements were made with a Hitachi 124 uv-visible spectrophotometer equipped with a thermostatted cell compartment. A Toa digital pH meter HM-15A was em-

Table II
Titration and Viscosity of Copolymers

Copolymer	Composition, mol % PHA	VIm	AAm	Functional group	pK_a	n'	pK_{int}	$[\eta]$[d]
PHA-VIm-AAm-1	5	35	60	PHA[b]	9.02	2.09	8.06	0.22
				VIm[c]	5.12	1.53	5.66	
PHA-VIm-AAm-2	3	7	90	PHA[b]	9.06	1.01	9.04	0.53
				VIm[c]	6.54	1.02	6.55	
PHA-AAm[a]	10		90	PHA[b]	9.12	1.45	8.72	0.32
VIm-AAm		10	90	VIm[c]	5.95	1.46	6.37	1.00

[a] Reference 12. [b] Potentiometric titration: 30 v/v % EtOH–H_2O, 30°, μ = 0.1 (KCl). [c] Uv titration: 28.9 v/v % EtOH–H_2O, 30°, μ = 0.1 (KCl), 0.15 M Barbital buffer. [d] Viscosity measurement: 1.0 M aqueous KCl, 30°.

Table III
Catalytic Hydrolysis by PHA-VIm-AAm-1[a]

Series	pH	k_{total}[b] × 10^5, sec^{-1}	k_{spont} × 10^5, sec^{-1}	k_{VIm},[c] M^{-1} sec^{-1}	k_{VIm}[VIm] × 10^5, sec^{-1}	$k_{a,obsd}$[PHA][d] × 10^5, sec^{-1}	$k_{a,obsd}$, M^{-1} sec^{-1}
A[e]	8.55	221	4.19	0.065	65.4	151	1.05
	8.74	355	7.04	0.081	80.8	267	1.86
	9.28	555[g]	10.0	0.092	92.4	453	3.15
B[f]	8.55	12.0	4.19	0.065	2.65	5.16	0.892
	8.74	19.5	7.04	0.081	3.27	9.19	1.57
	9.28	33.4	10.0	0.092	3.74	19.7	3.40

[a] 28.9 v/v % EtOH-H_2O, 30°, μ = 0.1 (KCl), 0.15 M Barbital buffer. [b] Rate constants determined by the Guggenheim procedure. [c] Estimated by the hydrolysis of PNPA by a VIm-AAm copolymer under otherwise the same reaction condition. [d] $k_{a,obsd}[PHA] = k_{total} - k_{spont} - k_{VIm}[VIm]$. [e] [PNPA] = 9.25 × 10^{-5} M, [PHA] = 1.43 × 10^{-3} M, [VIm] = 1.00 × 10^{-2} M. [f] [PNPA] = 1.16 × 10^{-4} M, [PHA] = 5.81 × 10^{-5} M, [VIm] = 4.07 × 10^{-4} M. [g] Determined by the first-order plot.

ployed for the pH measurement and a Toa pH stat system was used for the potentiometric titration. NMR spectra were obtained with a Varian A60 instrument. The polymer viscosity was measured by using a modified Ubbelohde viscometer.

Results

Catalytic Hydrolysis at Low Substrate Concentrations. The catalytic hydrolysis of PNPA by a bifunctional polymer catalyst was first conducted by using ordinary concentration ranges (10^{-4}–10^{-5} M) of PNPA. The total rate is composed of the catalytic and spontaneous terms. The polymer contains two kinds of the nucleophilic functional group: hydroxamate and imidazole. Therefore, the v_{cat} term consists of those due to the hydroxamate and imidazole functions.

$$v_{total} = v_{cat} + v_{spont}$$
$$= v_{PHA} + v_{VIm} + v_{spont} \qquad (2)$$

In series A experiments of Table III, large excesses of the catalytic groups are present compared to substrate, and the time course of the p-nitrophenol release necessarily follows the pseudo-first-order kinetics. The rate constant of phenol release due to the PHA unit, $k_{a,obsd}$, was determined by subtracting the spontaneous, k_{spont}, and imidazole, k_{VIm}, terms from the overall rate constant, k_{total}. The k_{VIm} value was determined separately from the hydrolysis of PNPA in the presence of a VIm-AAm copolymer (cf. Table II). The reactivity of the imidazole group in these copolymers can be assumed to be the same, as shown below.

In series B experiments, the concentration of the PHA catalytic group was lower than that of substrate. The time course of the phenol release followed the first-order kinetics, as proved by the linearity of the Guggenheim plot up to 80–90% completion of reaction (2–3 half-lives). The fact that the hydrolysis follows the first-order kinetics instead of the second-order kinetics indicates that the concentration of the catalytic group is kept constant during the reaction, i.e., the turnover of the catalytic group.

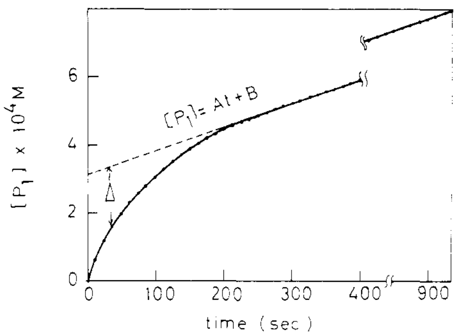


Figure 1. Time course of the burst-type p-nitrophenol release. Reaction condition: 28.9 v/v % EtOH–H_2O, 30°, μ = 0.1 (KCl), 0.15 M Barbital buffer. Catalyst: PHA-VIm-AAm-1, [PHA] = 4.03 × 10^{-4} M, [VIm] = 2.82 × 10^{-3} M, [PNPA] = 3.67 × 10^{-2} M.

The $k_{a,obsd}$ values obtained under these two reaction conditions agreed with each other reasonably well at the respective pH's. The relative efficiency of the PHA and VIm units in the phenol release may be estimated by a comparison of the $k_{a,obsd}$[PHA] and k_{VIm}[VIm] terms. The $k_{a,obsd}$[PHA] term is 2.5 to 5 times greater than the k_{VIm}[VIm] term in the present system.

Catalytic Hydrolysis at High Substrate Concentrations (Burst Kinetics). The release of p-nitrophenol in the reaction of a bifunctional polymer catalyst PHA-VIm-AAm with large excesses of PNPA shows typical burst kinetics, the initial rapid liberation of p-nitrophenol followed by the slower, steady release. An example is given in Figure 1. This curve is obtained by subtracting the phenol release due to spontaneous hydrolysis from the overall release. The experiments with and without a polymer catalyst were always conducted consecutively, in order to minimize the experimental error.

The analysis of the burst kinetics of simple nucleophilic catalysis has been worked out by Bender and Marshall.[15] In the present polymeric system, however, there are two

Table IV
Burst Experiment[a]

$[\text{PNPa}]_0 \times 10^2$, M	$A' \times 10^6$, M sec^{-1}	$b \times 10^2$, sec^{-1}	$B \times 10^4$, M	$k_{a,obsd}$,[b] M^{-1} sec^{-1}	$k_{d,obsd}$,[b] $\times 10^2$, sec^{-1}	k_{VIm},[b] M^{-1} sec^{-1}
1.84	7.54	3.62	2.36	1.25	1.36	0.05
2.75	8.67	4.61	2.51	1.18	1.35	0.04
3.21	11.1	5.49	2.65	1.24	1.50	0.05
3.67	12.1	5.28	2.68	1.05	1.40	0.05

[a] Polymer catalyst; PHA-VIm-AAm-1, $[\text{PHA}] = 5.03 \times 10^{-4}$ M, $[\text{VIm}] = 3.52 \times 10^{-3}$ M. Reaction condition, 28.9 v/v % EtOH–H$_2$O, 30°C, pH 8.55, $\mu = 0.1$ (KCl), 0.15 M Barbital buffer. [b] $k_{a,obsd}$, $k_{d,obsd}$, and k_{VIm} values obtained from eq 14, 15, and 12, respectively.

nucleophilic catalytic groups which contribute to the *p*-nitrophenol release. Therefore, a modification of the Bender procedure becomes necessary.

The nucleophilic catalysis of the ester hydrolysis is expressed by

$$\text{PHA} + \text{S} \xrightarrow[\text{fast}]{k_a} \underset{\displaystyle +\atop \displaystyle \text{P}_1}{\text{acetyl-PHA}} \xrightarrow[\text{slow}]{k_d} \text{PHA} + \text{P}_2 \qquad (3)$$

for the PHA group, and by

$$\text{VIm} + \text{S} \xrightarrow[\text{slow}]{k_{\text{VIm}}} \underset{\displaystyle +\atop \displaystyle \text{P}_1}{\text{acetyl-VIm}} \xrightarrow[\text{fast}]{} \text{VIm} + \text{P}_2 \qquad (4)$$

for the VIm group, where P_1 and P_2 denote *p*-nitrophenol and acetic acid, respectively.

The acetyl intermediate was not detectably accumulated in the imidazole-catalyzed hydrolysis of PNPA in the present system, since the catalytic hydrolysis by the VIm-AAm copolymer followed the first-order kinetics.

The formation of *p*-nitrophenol (P_1) at time t according to eq 3 is given by the following equation, if $[\text{PHA}]_0 \ll [\text{S}]_0$.[15]

$$[\text{P}_1]_{\text{PHA}} = At + B(1 - e^{-bt}) \qquad (5)$$

where

$$A = \frac{k_a k_d [\text{S}]_0 [\text{PHA}]_0}{k_a[\text{S}]_0 + k_d} \qquad (6)$$

$$B = \frac{k_a^2 [\text{S}]_0^2 [\text{PHA}]_0}{(k_a[\text{S}]_0 + k_d)^2} \qquad (7)$$

$$b = k_a[\text{S}]_0 + k_d \qquad (8)$$

and

$$[\text{acetyl-PHA}] = \frac{k_a[\text{S}]_0[\text{PHA}]_0}{k_a[\text{S}]_0 + k_d}(1 - e^{-bt}) \qquad (9)$$

On the other hand, the *p*-nitrophenol release due to the VIm group (eq 4) is given at the early stage of reaction by

$$[\text{P}_1]_{\text{VIm}} = k_{\text{VIm}}[\text{S}]_0[\text{VIm}]_0 t \qquad (10)$$

The total release is the sum of eq 5 and 10.

$$[\text{P}_1] = A't + B(1 - e^{-bt}) \qquad (11)$$

where

$$A' = \frac{k_a k_d [\text{S}]_0 [\text{PHA}]_0}{k_a[\text{S}]_0 + k_d} + k_{\text{VIm}}[\text{S}]_0[\text{VIm}]_0 \qquad (12)$$

Δ is defined as the difference in $[\text{P}_1]$ between the dotted line (extrapolation of the steady portion) and the burst curve (Figure 1).

$$\Delta = Be^{-bt} \qquad (13)$$

The B and b values are obtained from the intercept and slope, respectively, of the linear plot of log Δ and t, and k_a and k_d values are determined from the following equations.

$$k_a = b\sqrt{B}/[\text{S}]_0\sqrt{[\text{PHA}]_0}, \qquad (14)$$

$$k_d = b - k_a[\text{S}]_0 \qquad (15)$$

The k_{VIm} value is calculated from eq 12 by using A' (slope of the linear portion), k_a, and k_d.

The effect of varying the substrate concentration on the burst kinetics is presented in Table IV. High substrate concentrations (2–4×10^{-2} M) were used. All of the k_a, k_d, and k_{VIm} terms are included in the steady-state release (A') and they cannot be simultaneously determined from eq 12. Thus, the k_a and k_d values were calculated from the presteady state using B and b values which were obtained by the logarithmic plot of eq 13. Then, k_{VIm} was calculated using A', k_a, and k_d values (eq 12). The agreements of the rate constants among different runs are fair. Alternatively, the b value obtained was plotted against $[\text{S}]_0$ according to eq 8, and $k_{a,obsd}$ and $k_{d,obsd}$ were determined to be 1.24 M^{-1} sec^{-1} and 1.60×10^{-2} sec^{-1}, respectively. The correlation coefficient of the least-squares method for the b–$[\text{S}]_0$ linear relation was 0.910.

The turnover rate A of the PHA unit at the steady state (eq 6) is calculated from A' and k_{VIm} by using eq 12, and the double reciprocal plot of eq 6 gives k_a and k_d values. The $k_{a,obsd}$ and $k_{d,obsd}$ values thus determined from the data of Table IV were 1.16 M^{-1} sec^{-1} and 1.42×10^{-2} sec^{-1} ($r = 0.93$), respectively. The agreements of $k_{a,obsd}$ and $k_{d,obsd}$ obtained by three different methods (eq 14 and 15, eq 8 and eq 6) are satisfactory and it is concluded that the present burst experiment and its kinetic analysis are reasonably reliable. In the following the $k_{a,obsd}$ and $k_{d,obsd}$ values are determined preferentially from the presteady state data because of their better accuracy.

Subsequently, the burst experiment was conducted by using PHA-VIm-AAm-1 copolymer at several pH's. The results are summarized in Table V. The $k_{a,obsd}$ and $k_{d,obsd}$ values were determined by using eq 14 and 15. α_{PHA} is the fraction of the dissociated PHA unit at the respective pH, and calculated from the titration data of Table II and the modified Henderson–Hasselbach equation (eq 1). α_{VIm}, the fraction of the neutral VIm unit, was similarly calculated. The $k_{a,obsd}$ value increases with increasing pH, reflecting the fact that the hydroxamate anion is the true reacting species. The k_{VIm} value cannot be determined in sufficient accuracy from the burst experiment (eq 12). However, k_{VIm} thus determined shows satisfactory agreements with those determined more accurately from the reaction of the VIm-AAm copolymer with PNPA (cf. Table III). For instance, $k_{\text{VIm}} = 0.05$ M^{-1} sec^{-1} at pH 8.56 in the burst kinetics and $k_{\text{VIm}} = 0.065$ M^{-1} sec^{-1} at pH 8.55 in the catalytic hydrolysis with the VIm-AAm copolymer.

The burst experiment was also performed with the PHA-VIm-AAm-2 copolymer which contains smaller amounts of the catalytic groups. The concentrations of the PHA unit

Table V
Burst Experiment[a]

pH	$k_{a,obsd}$, M^{-1} sec^{-1}	α_{PHA}[b]	$k_{d,obsd} \times 10^2$, sec^{-1}	k_{VIm},[c] M^{-1} sec^{-1}
8.04	0.79	0.253	0.963	0.03
8.35	1.25	0.323	1.31	0.02
8.56	1.69	0.376	1.69	0.05
8.80	2.29	0.440	2.55	0.09
9.01	2.59	0.497	2.64	0.08
9.18	3.47	0.544	2.76	0.09

[a] Reaction condition: 28.9 v/v % EtOH–H$_2$O, 30°, μ = 1.0 (KCl), 0.15 *M* Barbital. Catalyst: PHA-VIm-AAm-1, [PHA] = 5.81×10^{-4} *M*, [VIm] = 4.08×10^{-3} *M*. Substrate: (1.35–3.70) $\times 10^{-2}$ *M*. [b] $\alpha_{PHA} = 1/[1 + 10^{(9.02\text{-pH})/2.09}]$. [c] α_{VIm} = 0.99 — 1.00.

Table VI
Reaction of Acetyl Hydroxamates and Imidazole[a]

Acetyl hydroxamate	pH	[acetyl PHA] $\times 10^4$, *M*	$k^b \times 10^3$, M^{-1} sec^{-1}
Acetyl derivative of model PHA	8.16	17.3	7.17
	8.40	17.3	6.80
	9.03	10.4	7.30
	9.46	6.93	7.34
Acetylated PHA-AAm copolymer	8.09	8.61	4.79
	8.43	8.61	4.87
	8.92	4.41	4.81[c]
	9.30	4.41	5.01[c]

[a] Imidazole, 0.960 *M*; 30°; 28.9 v/v % EtOH-H$_2$O; μ = 0.1 (KCl); 0.15 *M* Barbital buffer. [b] $k = k_{obsd}/[\text{imidazole}]_{total}$ [c] Imidazole, 0.853 *M*.

and PNPA in these reaction mixtures were (3.85–7.72) $\times$ 10^{-4} *M* and (9.38–18.9) $\times 10^{-3}$ *M*, respectively. The kinetic constants were similarly determined, except for the fact that the k_{VIm} term was too small to be determined from eq 12. This is caused by the small content (7 mol %) of the VIm unit in this copolymer. Smaller $k_{d,obsd}$ values ((0.06–1.0) $\times 10^{-2}$ sec^{-1}) observed for this polymer relative to those for PHA-VIm-AAm-1 are also related to the decreased VIm content. These data are given in the figures shown below.

Hydrolysis of Acetyl Hydroxamate by Imidazole. The imidazole-catalyzed hydrolysis of polymeric and small-molecule acetyl hydroxamates was carried out and the results are summarized in Table VI. The reaction as measured by the appearance of the hydroxamate anion was pseudo-first order, and the rate constant k_{obsd} was determined by the Guggenheim procedure up to 2–3 half-lives. The spontaneous term was negligible. It has been shown by Kirsch and Jencks that the catalytic hydrolysis of *N,O*-diacetyl-*N*-methylhydroxylamine by imidazole proceeds by the nucleophilic mechanism.[16] The same mechanism would probably hold for the present system. The rate constants observed are smaller by one order than the figure obtained by Kirsch and Jencks for *N,O*-diacetyl-*N*-methylhydroxylamine at 25° in an aqueous solution.

Discussion

Scheme I is conceived for the hydrolysis of PNPA by the bifunctional polymer.

The terpolymer contains two kinds of the nucleophilic functions, PHA and VIm units. It is shown from a comparison of $k_{a,obsd}$ and k_{VIm} that the PHA unit is much more reactive than the VIm unit in the pH range studied (cf. Tables III and V). Therefore, the predominant course of catalysis is the acyl transfer from PNPA to the PHA anion and the subsequent hydrolysis of the acetyl hydroxamate intermediate.

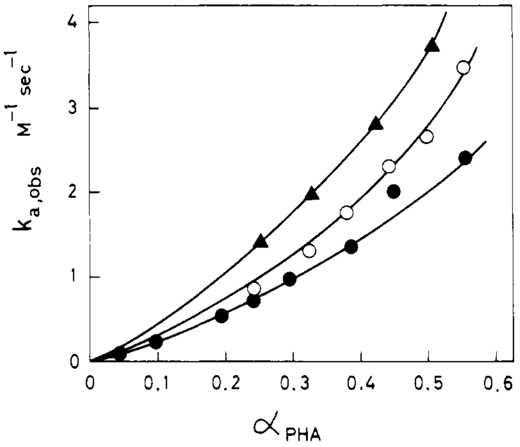


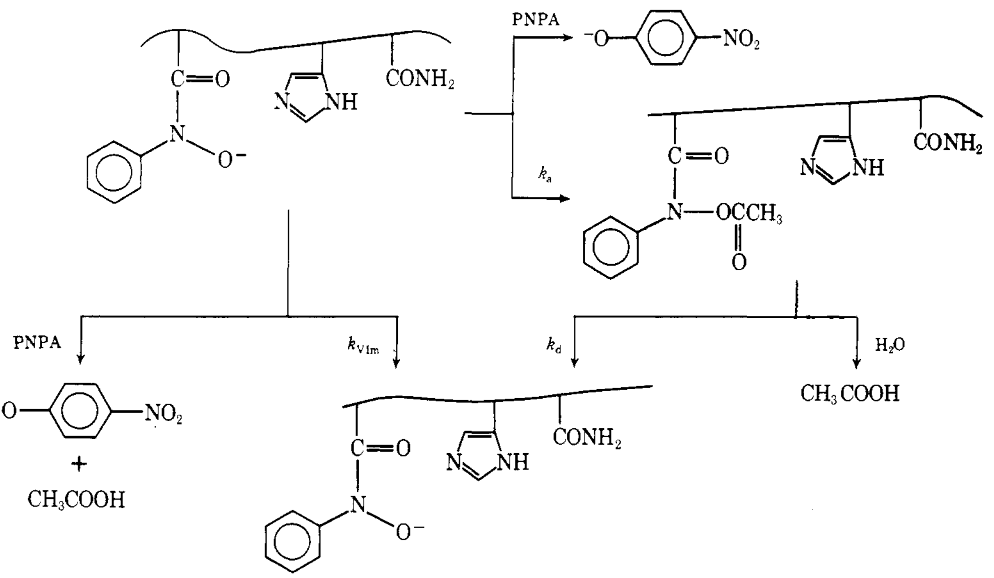
Figure 2. Acetylation of hydroxamate copolymers by PNPA. Reaction condition: 28.9 v/v % EtOH-H$_2$O, 30°, μ = 0.1 (KCl), 0.15 *M* Barbital buffer. α_{PHA}: fraction of the dissociated PHA unit. Copolymer: (▲) PHA-AAm (pK_a = 9.12, n' = 1.45, pK_{int} = 8.72) cf. ref 7; (○) PHA-VIm-AAm-1; (●) PHA-VIm-AAm-2.

At the commonly employed range of the PNPA concentration (10^{-4}–10^{-5} *M*), the *p*-nitrophenol release by PHA-VIm-AAm-1 followed the first-order kinetics, even when the concentration of the PHA unit was smaller than that of substrate (Table III, series B). This requires that there is no accumulation of the acetylated PHA unit and that the PHA unit is employed repeatedly as the site of acylation during the hydrolysis. In the reaction of a PHA-AAm copolymer with PNPA under similar conditions, the deacylation process is very sluggish compared with acylation, and the acetyl hydroxamate group is accumulated. Therefore, the present kinetic data are explained by assuming faster deacylation due to the intramolecular imidazole catalysis. It is of particular importance that the rate-determining step of the nucleophilic catalysis can be changed from deacylation to acylation even for a powerful nucleophile like hydroxamate anions.

Acylation Process. Figure 2 shows the relation between the apparent rate constant of acylation at the PHA unit, $k_{a,obsd}$ and the fraction of the dissociated PHA unit α. The $k_{a,obsd}$ – α_{PHA} relations for small-molecule hydroxamic acids are linear, reflecting the fact that the hydroxamate anion is the true reacting species.[12,17] On the other hand, upward curvatures with α were observed in the polymeric system. The same tendency has been found for other PHA-containing copolymers.[7,12] As discussed previously, at small extents of dissociation, the reactivity of a given hydroxamate anion may be lessened because of its intramolecular aggregation with the undissociated PHA unit. With increasing degrees of dissociation, the side-chain aggregation will be destroyed by expansion of the polymer coil due to increasing electrostatic repulsion among the negatively charged groups. This interpretation is consistent with the deviation of n' from unity (Table II) which implies the interaction of the charged groups.

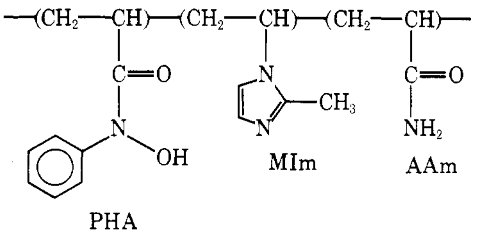
It is seen from Figure 2 that the reactivity of the PHA anion is greater for the PHA-AAm copolymer than for the PHA-VIm-AAm copolymers. It was expected earlier that there might arise an enhanced efficiency of acylation due to the cooperative action of the PHA group (neutral and/or anionic species) and the neighboring imidazole group, as in the charge relay system of serine proteases. However, introduction of the VIm unit seems to simply decrease the nucleophilic reactivity of the PHA anion, because of the in-

Scheme I



creased aggregation of the side chains. The lack of the cooperative action may also be supported by the fact that the k_{VIm} values obtained for PHA-VIm-AAm-1 and VIm-AAm polymers agreed within the experimental error. It appears that the presence of the PHA unit does not appreciably alter the reactivity of the VIm unit, either.

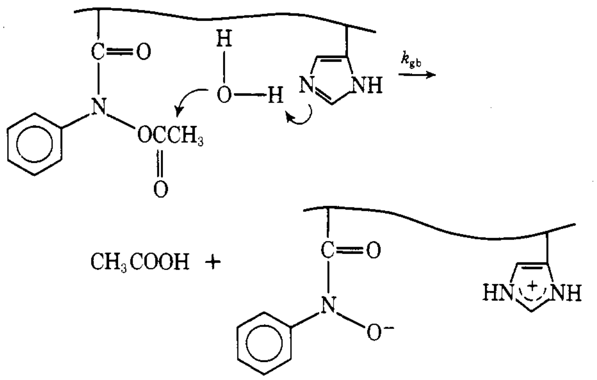
Deacylation Process. In Figure 3 are plotted log $k_{d,obsd}$ against pH. The corresponding plots for a PHA-MIm-AAm polymer[7] are included in the figure.



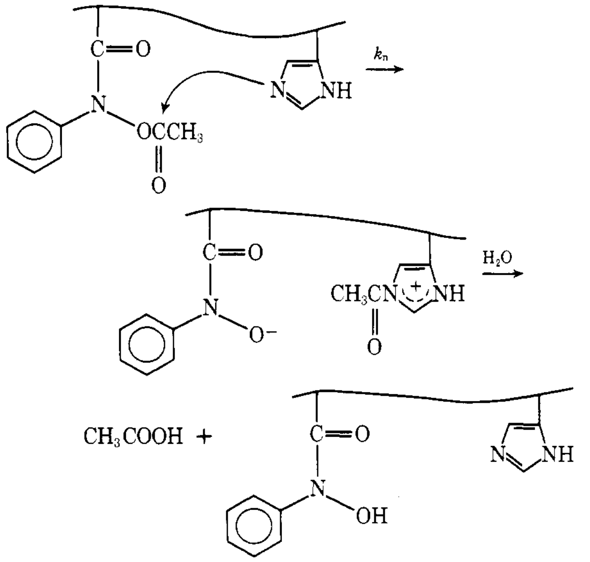
The rate constants of the spontaneous hydrolysis of several acetyl hydroxamate compounds[7] are similarly plotted for comparison. It is clear that $k_{d,obsd}$ is enhanced considerably by introduction of the VIm unit. The rate enhancement between PHA-VIm-AAm-1 and PHA-AAm amounted to 10^3-fold at pH 8.5 (1.69×10^{-2} sec^{-1} vs. 1.70×10^{-5} sec^{-1}, cf. Figure 3). The $k_{d,obsd}$ values for PHA-VIm-AAm-1 (VIm content 35 mol %) were greater than those for PHA-VIm-AAm-2 (VIm content, 7 mol %) by a factor of 3–10 over the pH range studied. This reflects the different efficiency of intramolecular imidazole catalysis.

The log $k_{d,obsd}$ value was proportional to pH (slope = 1) for PHA-AAm and model PHA, which indicates the occurrence of alkali hydrolysis. The bifunctional polymers behave differently. The log $k_{d,obsd}$ value increases with pH up to ca. 8.8, and then levels off. The increase is pronounced at pH 8.4 to 8.8. The log $k_{d,obsd}$ value for PHA-MIm-AAm is proportional to pH with a slope of 0.8. This linear relation could be associated with the logarithmic decrease of the positive charge in the polymer chain, and the consequent conformational change.[7]

The imidazole group can accelerate deacylation in two ways: general basic and nucleophilic catalyses. In the general base catalysis, the VIm unit helps H_2O attack on the acetyl hydroxamate (eq 16). The nucleophilic catalysis is constituted of the acetyl transfer from the PHA unit to the VIm unit and the hydrolysis of the acetylated VIm unit. At present, there is no direct evidence to discriminate these two possibilities. However, it should be pointed out that Kirsch and Jencks showed that the imidazole-catalyzed hydrolysis of *N,O*-diacetyl-*N*-methylhydroxylamine proceeds by the nucleophilic mechanism.[16]



(16)



(17)

In either case, the deacylation is effected by the basic VIm unit. The majority of the VIm unit exists as the neutral species above pH 8 because of its low pK_a values. Thus, the $k_{d,obsd}$ variation cannot be attributed to the simple availability of the neutral imidazole species. Two possibili-

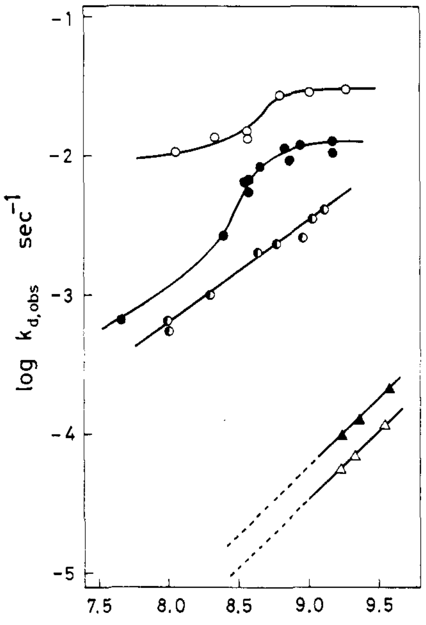
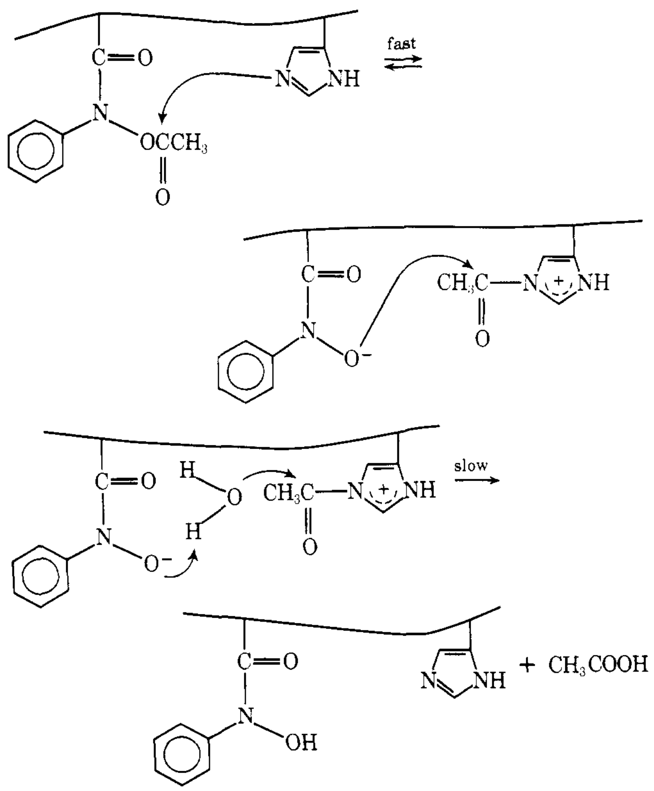


Figure 3. pH dependence of deacylation rates. Reaction condition: 28.9 v/v % EtOH-H_2O, 30°, μ = 0.1 (KCl), 0.15 *M* Barbital (○, ●, ◐). Lower plots (▲, △) were obtained by extrapolation to the zero buffer concentration. Copolymer: (○) PHA-VIm-AAm-1; (●) PHA-VIm-AAm-2; (◐) PHA-MIm-AAm-4 (from ref 7); (▲) acetyl-PHA-AAm; (△) acetylated model PHA.

ties are conceivable for explaining the observed $k_{d,obsd}$ variation.



(1) The conformation of the polymer chain changes with pH increase so as to render the assistance of deacylation by imidazole more effective at higher pH's.

(2) Assuming that the deacylation process involves the rapid, reversible acyl transfer to imidazole, the decomposition of the acetylimidazole intermediate can be the slow step of deacylation which is accelerated by the general base catalysis of the hydroxamate anion.

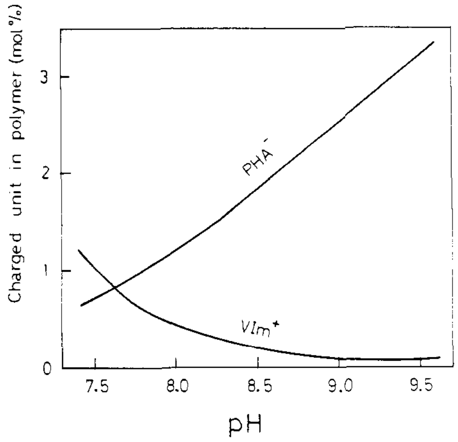


Figure 4. Variation of the content of the charged units in PHA-VIm-AAm-1. Solid curves are calculated from the copolymer composition and the degree of dissociation. The content of the charged units is given relative to the total monomer unit.

Figure 4 is the plot of the content of the charged units (PHA$^-$ and VIm$^+$) against pH. The content of the charged species is quite small at pH 7.5 to 9.5, because the VIm unit is mostly in the neutral form. The isoelectric point is 7.62. Thus, it is difficult to assume that the change of the polymer conformation based on the electrostatic interaction is the major factor of the $k_{d,obsd}$ variation.

Interestingly enough, the region of the steep rise of $k_{d,obsd}$ corresponds roughly to the dissociation of the PHA unit. This fact is consistent with the second possibility.

The acetylimidazole group formed by acetyl transfer may also be hydrolyzed by hydroxide ion or by another VIm unit. The rate constant of water hydrolysis of acetylimidazole was reported by Jencks[18] to be (0.3–1.5) × 10^{-3} sec^{-1} at 25°, pH 8–9. Overberger and coworkers studied the hydrolysis of partially acetylated poly(4(5)-vinylimidazole) ($k_{d,obsd}$ = (2.3–4) × 10^{-3} sec^{-1} in 20–40% aqueous alcohol at 26°, pH 8) and concluded that the general base catalysis of the neighboring VIm group enhanced the hydrolysis.[8] These rates for the decomposition of acetylimidazole in small-molecule and polymeric systems are smaller than $k_{d,obsd}$ found in this study: 0.01–0.03 sec^{-1} for PHA-VIm-AAm-1. Therefore, the predominant course of deacylation cannot be the hydrolysis of the acetyl VIm unit catalyzed by the neighboring VIm unit. Furthermore, this mechanism cannot explain the observed $k_{d,obsd}$ variation.

An important question remaining in the deacylation process is whether or not we are observing a true turnover process, i.e., is the catalytic site truly regenerated? In the present kinetic analysis, the turnover of the PHA unit is followed through *p*-nitrophenol release, and there is a possibility that the acetyl group is transfered from the PHA unit and accumulated on the VIm unit.

In the burst type hydrolysis with PHA-VIm-AAm-2, the steady catalytic release of *p*-nitrophenol (its rate being determined by the acetyl transfer) was observed, even after PNPA which corresponds to several equivalents of the PHA unit was hydrolyzed. This polymer contains 3 mol % PHA unit and 7 mol % VIm unit, and the acetyl group cannot be accumulated on the VIm unit not more than 2.3 equiv of the PHA unit. Thus, it is not probable that the acetyl VIm unit is accumulated to a considerable degree during the catalysis. The same can probably be said for PHA-VIm-AAm-1, which contains a greater amount of the

Table VII
Catalytic Efficiency of Polymer Catalysts at pH 8[a]

Catalyst	$k_{\text{turnover}} \times 10^5$ (sec^{-1}) at $[S]_0$ =			
	10^{-1} *M*	10^{-2} *M*	10^{-3} *M*	10^{-4} *M*
PHA-VIm-AAm-1	850	430	70	8
PHA-AAm	0.56	0.56	0.56	0.56
VIm-AAm	90	40	6	0.6

[a] 28.9 v/v % EtOH–H_2O, 30°, $\mu = 0.1$ (KCl).

VIm unit, although the *p*-nitrophenol release could not be followed much further.

Catalytic Efficiency. The efficiency of a catalytic system may be assessed most appropriately in terms of the turnover rate. The turnover rate is, by definition, equal to the rate of the catalyst regeneration.

$$v_{\text{turnover}} = k_{\text{d}}[\text{acetyl PHA}] \qquad (18)$$

From eq 9 and 19,

$$v_{\text{turnover}} = \frac{k_{\text{a}}k_{\text{d}}[\text{S}]_0}{k_{\text{a}}[\text{S}]_0 + k_{\text{d}}}[\text{PHA}]_0(1 - e^{-bt}) \qquad (19)$$

Under the steady-state condition (i.e., $t \rightarrow \infty$)

$$v_{\text{turnover}} = \frac{k_{\text{a}}k_{\text{d}}[\text{S}]_0}{k_{\text{a}}[\text{S}]_0 + k_{\text{d}}}[\text{PHA}]_0 \qquad (20)$$

$$v_{\text{turnover}} = k_{\text{turnover}}[\text{PHA}]_0 \qquad (21)$$

Thus, the apparent rate constant of turnover, k_{turnover}, is related to rate constants of acylation and deacylation by eq 20 and 21. The k_{turnover} value was calculated for the respective polymer catalysts at several substrate concentrations as shown in Table VII. The relative efficiency of turnover, acylation, and deacylation is estimated by comparing k_{turnover}, $k_{\text{a,obsd}}[\text{S}]_0$, and $k_{\text{d,obsd}}$. At low substrate concentrations (10^{-4}–10^{-5} *M*), $k_{\text{a,obsd}}[\text{S}]_0$ is much smaller than $k_{\text{d,obsd}}$ for the bifunctional polymer, PHA-VIm-AAm-1. This means that the acylation step is rate limiting. In fact, the kinetics of PNPA disappearance were first order, suggesting complete turnover of the catalytic group: $k_{\text{turnover}} = k_{\text{a,obsd}}[\text{S}]_0$. The k_{turnover} value for the PHA unit in PHA-AAm is virtually equal to $k_{\text{d,obsd}}$ (t.6 × 10^{-6} sec^{-1} at pH 8, cf. Figure 3), because acylation is much faster. On the other hand, k_{turnover} for the VIm unit in VIm-AAm polymer becomes equal to $k_{\text{VIm}}[\text{S}]_0$ (6×10^{-6} sec^{-1} at $[\text{S}]_0 = 10^{-4}$ *M*), as the acylation step is rate limiting. The catalytic efficiency of this bifunctional polymer is about ten times greater than the polymers containing either one of the two functions at pH 8, $[\text{S}]_0 = 10^{-4}$ *M*. The presence of complementary functional groups is undoubtedly advantageous in polymer catalysis.

At higher substrate concentrations, the acylation process becomes much more efficient for both of the PHA and VIm functions, and deacylation is rate limiting in most cases. The bifunctional polymer is 10 to 10^3 times more efficient than PHA-AAm or VIm-AAm at $[\text{S}]_0 = 0.1$ *M* (see Table VII).

The catalytic efficiency may also be discussed relative to the uncatalyzed rate of hydrolysis. Since the k_{spont} value at pH 8.0 is 2.6×10^{-5} sec^{-1}, v_{spont} becomes 2.6×10^{-6} *M* sec^{-1} at 0.1 *M* substrate. The v_{turnover} value for 1 *M* of PHA-VIm-AAm-1 under the same reaction condition is 8.50×10^{-3} *M* sec^{-1} (Table VII). Therefore, v_{turnover} is more than 3000 times greater than v_{spont}. In other words, less than $\frac{1}{3000}$ *M* of the catalyst is required for effecting the hydrolysis of PNPA in a rate comparable to that of the spontaneous hydrolysis. Similarly, v_{turnover} for 1 *M* catalyst is 3×10^4 times greater than v_{spont} at 10^{-4} *M* PNPA.

The major role of the imidazole group is *intramolecular* catalysis of the decomposition of the acetyl hydroxamate intermediate. Table VI contains the results on the decomposition of the acetyl hydroxamate by *intermolecular* imidazole catalysis. The second-order rate constants of the latter reaction are ca. 7×10^{-3} M^{-1} sec^{-1} for the small-molecule derivative and ca. 5×10^{-3} M^{-1} sec^{-1} for the polymeric derivative. On the other hand, $k_{\text{d,obsd}}$ is (1–3) × 10^{-2} sec^{-1} for polymer in the same pH range. Therefore, the relative rate constant becomes 2–5 *M* between the *intramolecular* and *intermolecular* imidazole catalyses. This is not a remarkable figure, in view of much greater ratios (up to 10^8 *M*) found for the nucleophilic attack of the carboxylate group.[19] Probably, the spatial arrangement of the two functional groups is not very proper because of the flexibility of the polymer chain.

The catalytic efficiency of the bifunctional system may be further enhanced by modifications of catalysts and by using proper combinations of catalyst and substrates. Recently, several groups of investigators have shown that the reactivity of the hydroxamate and oximate anions is remarkably increased in polymeric[20,21] and micellar[11,22,23] systems. The deacylation step may be largely determined by the steric arrangement of the two functional groups and their environments. Actually we observed a wide variation of deacylation rates for a series of small molecules containing hydroxamate and imidazole functions.[24] Therefore, we can look forward to synthesizing much more efficient catalytic systems for ester hydrolysis along the line of the present research.

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